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(54) Title: NON-STOCHASTIC GENERATION OF GENETIC VACCINES AND ENZYMES

(57) Abstract: This invention provides methods of obtaining novel polynucleotides and encoded polypeptides by use of non-stochastic methods of directed evolution (DirectEvolutionTM). These methods include non-stochastic polynucleotide site-saturation mutagenesis (Gene Site Saturation MutagenesisTM) and non-stochastic polynucleotide reassembly (GeneReassemblyTM). Through use of the claimed methods, genetic vaccines, enzymes, and other desirable molecules can be evolved towards desirable properties. For example, vaccine vectors can be obtained that exhibit increased efficacy for use as genetic vaccines. Vectors obtained by using the methods can have, for example, enhanced antigen expression, increased uptake into a cell, increased stability in a cell, ability to tailor an immune response, and the like. This invention provides methods of obtaining novel enzymes that have optimized physical and/or biological properties. Furthermore, this invention provides methods of obtaining a variety of novel biologically active molecules, in the fields of antibiotics, pharmacotherapeutics, and transgenic traits.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/03086**A. CLASSIFICATION OF SUBJECT MATTER**IPC(7) : C12Q 1/68; C12N 15/09; C07H 21/02; A61K 39/00
US CL : 435/6, 69.3; 536/23.1; 424/184.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 69.3; 536/23.1; 424/184.1; 435/7.6, 69.1, 69.7; 530/350

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

DIALOG, MEDLINE, EMBASE, BIOSIS, US PATENTS, immunomodulatory polynucleotide, library, non-stochastic, mutagenesis, genetic vaccine

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4,959,312 A (SIROTKIN) 25 September 1990, Abstract.	1-85
Y	CWIRLA et al. Peptides on phage: A vast library of peptides for identifying ligands, Proc. Natl. Acad. Sci. USA. August 1990, Vol. 87, pages 6378-6382, especially page 6378, Materials and Methods.	1-85
Y	HILL et al. Mutagenesis with Degenerate oligonucleotides: An Efficient Method for Saturating a Defined DNA Region with Base Pair Substitutions. Methods in Enzymology. 1987, Vol. 155, pages 558-568, especially pages 561-563.	1-85

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/03086

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category ^a	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MURRAY et al. Saturation mutagenesis of a major histocompatibility complex protein domain: Identification of a single conserved amino acid important for allorecognition. Proc. Natl. Acad. Sci. USA. May 1988, Vol. 85, pages 3535-3539, especially pages 3535-3536.	1-85